Turner JG, Novacky A (1974) The quantitative relation between plant and bacterial cells involved in the hypersensitive reaction. Phytopathology 64: 885-90

Van Gijsegem F (1906) Analysis of the pectin-degrading enzymes secreted by three strains of Erwinia chrysanthemi. J Gen Microbiol 132: 617-624

Van Gijsegem F (1989) Relationship between the pel genes of the pelADE cluster in Erwinia chrysanthemi strain B374. Mol Microbiol 3: 1415-1424

Van Gijsegem F, Genin S, Boucher C (1993) Evolutionary conservation of pathogenicity determinants among plant and animal pathogenic bacteria. Trends Microbiol 1: 175-180

Vayda ME, Scheeller HJ (1988) Hypoxic stress inhibits the appearance of wound-response proteins in potato tubers. Plant Physiol 88: 805-809

Vayda ME, Antonov LS, Yang Z, Butler WO, Lacy GH (1992) Hypoxic stress inhibits aerobic wound-induced resistance and activates hypoxic resistance to bacterial soft rot. Am Potato J 69: 239-253

Wandersman C (1989) Secretion, processing and activation of bacterial extracellular proteases. Mol Microbiol 3: 1825-1831

Wandersman C, Delepelaire P, Letoffe S, Schwartz M (1987) Characterization of Erwinia chrysanthemi extracellular proteases: cloning and expression of the protease genes in Escherichia coli. J Bacteriol 169: 5046-5053

Wei Z-M, Laby RJ, Zumoff CH, Bauer DW, Ho SY, Collmer A, Beer SV (1992) Harpin, elicitor of the hypersensitive response produced by the plant pathogen Erwinia amylovora. Science 257: 85-88

Whalen MC, Inness RW, Bent AF, Staskawicz BJ (1991) Identification of Pseudomonas syringae pathogens of Arabidopsis and a bacterial locus determining avirulence on both Arabidopsis and soybean. Plant Cell 3: 49-59

Willis DK, Barta TM, Kinscherf (1991a) Genetics of toxin production and resistance in phytopathogenic bacteria. Experientia 47: 765-771

Willis DK, Rich JJ, Hrabak EM (1991b) hrp genes of phytopathogenic bacteria. Mol Plant Microbe Interact 4: 132-138

Xiao Y, Lu Y, Heu S, Hutcheson SW (1992) Organization and environmental regulation of the Pseudomonas syringae pv. syringae 61 hrp cluster. J Bacteriol 174: 1734-1741

Yamazaki N, Fry SC, Darvill AG, Albersheim P (1983) Host-pathogen interactions. XXIV. Fragments isolated from suspension-cultured sycamore cell walls inhibit the ability of the cells to incorporate [14C] leucine into proteins. Plant Physiol 72: 864-869

Yang Z, Cramer CL, Lacy GH (1992) Erwinia carotovora subsp. carotovora pectic enzymes: in planta gene activation and roles in soft-rot pathogenesis. Mol Plant Microbe Interact 5: 104-112

Yoder MD, Keen NT, Jurnak F (1993) New domain motif: the structure of pectate lyase C, a secreted plant virulence factor. Science 260: 1503-1507

Young JM (1974) Development of bacterial populations in vivo in relation to plant pathogenicity. NZ J Agric Res 17: 105-113

Yucel I, Xiao Y, Hutcheson SW (1989) Influence of Pseudomonas syringae culture conditions on initiation of the hypersensitive response of cultured tobacco cells. Appl Environ Microbiol 55: 1724-1729

Zink RT, Engwall JK, Chatterjee AK (1985) recA is required in the induction of pectin lyase and carotovoricin in Erwinia carotovora subsp. carotovora. J Bacteriol 164: 390-396

Zink RT, Hankin L (1970) Regulation of pectate lyase synthesis in Pseudomonas fluorescens and Erwinia carotovora. J Bacteriol 104: 13-18

Zucker M, Hankin L, Sands D (1972) Factors governing pectate lyase synthesis in soft rot and non-soft rot bacteria. Physiol Plant Pathol 2: 59-67

hrp Genes of Phytopathogenic Bacteria

U. Bonas

1 Introduction

In nature plants are resistant to the majority of pathogens, and many bacteria live in close contact with the plant without causing any harm (see chapter by BEATTIE and LINDOW in this volume). Among the 1600 different species known in the bacterial kingdom only a small number (about 80) are plant pathogenic and in most cases highly specialized with respect to the plant that can be attacked. Only a few of these species are gram-positive, e.g., Clavibacter ssp. and Streptomyces ssp. In this review I focus on subspecies of the gram-negative genera Erwinia, Pseudomonas, and Xanthomonas, which comprise the major bacterial plant pathogens.

To be a successful pathogen the invading bacterium has to overcome the plant's defense. During evolution plant pathogenic bacteria have acquired multiple functions that enable them to colonize and multiply in living plant tissue. In nature, bacteria enter the plant through natural openings (stomata, hydathodes) or

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wounds. The bacterial armory contains a number of weapons that contribute to pathogenicity. Obvious examples include degradative extracellular enzymes such as pectinases, cellulases, and proteases. When the corresponding genes are mutated, bacterial ability to invade plant tissues is more or less affected depending on the pathogen, i.e., these functions contribute to and modulate development and severity of infection to different extents (see chapters by Dow and Daniels, and Collmer and Bauer in this volume).

In addition, phytopathogenic bacteria possess a large number of genes needed for basic pathogenicity. These genes have been operationally defined as *hrp* (hypersensitive reaction and pathogenicity; Lindgren et al. 1986) based on their mutant phenotype. *hrp* genes are not only essential for pathogenicity on a plant, i.e., the ability to cause disease in a compatible interaction, but also for the incompatible interaction with resistant host varieties or with plants that are not normally a host for the particular pathogen (so called non-host). The incompatible interaction is often associated with the induction of a hypersensitive reaction (HR) in the plant. In contrast to the use of the term hypersensitivity in the animal field, the HR is a rapid defense response involving localized plant cell death, in plants the HR is a rapid defense response involving localized plant cell death, production of phenolics and antimicrobial agents, e.g., phytoalexins, at the site of infection (Klement 1982; Lindsay et al. 1993). The HR results in prevention of pathogen multiplication and spread and thus in prevention of disease development. Under natural infection conditions the HR is microscopically small and can be induced by just one bacterial cell. Only when bacteria are introduced into plant tissue at high cell densities in the laboratory (about 10^7 colony forming units or more/ml) is the HR macroscopically visible as confluent necrosis and can be clearly distinguished from typical disease symptoms. It is important to note that saprophytic or nonpathogenic bacteria such as *Escherichia coli* or *Pseudomonas fluorescens* do not induce the HR and are unable to multiply in plant tissue.

2 Isolation of *hrp* Genes and General Features

hrp genes have been isolated from all major gram-negative plant pathogenic bacteria except Agrobacterium. There are excellent reviews that describe the early work or locus more on one particular pathogen (Willis et al. 1991; Boucher et al. 1992). The majority of *hrp* genes have been identified by complementation of loss-of-function mutants. Mutants obtained by random chemical (e.g. N-methyl-N'-nitro-N-nitrosoguanidine) or transposon mutagenesis of a pathogenic wild-type strain were inoculated into the host plant and screened for loss of both the ability to cause disease in susceptible plants and to induce the HR in resistant host or non-host plants (often tobacco). The second criterion for the isolation of genes specific for the plant interaction was to ensure that the mutants would still grow in minimal medium. This way mutants affected in genes for basic housekeeping functions were eliminated. A third characteristic of all *hrp* mutants is that they are unable to grow in the plant.

The *hrp* genes were originally described f r the bean pathogen *Pseudomonas syringae* pv. *phaseolicola*. Lindgren and coworkers (1986) isolated Tn5-induced mutants of *P.s.* pv. *phaseolicola* that had lost both the ability to induce halo-blight disease on bean and the HR in tobacco. Complementation with cosmid clones from a genomic library of the wild-type strain resulted in isolation of a cluster of *hrp* genes localized in a 20 kb DNA region. This was the first indication that both the ability to cause disease and to induce the HR are mediated by common steps in a "pathway".

Since then *hrp* gene clusters have been cloned from a number of different bacteria. Examples include *Pseudomonas solanacearum* (Boucher et al. 1987; Fig. 1B); the *Xanthomonas campestris* pathovars *campestris* and *vitians* (Arlat et al. 1991), *translucens* (Waney et al. 1991), and *vesicatoria* (Bonas et al. 1991; Fig. 1A), *Erwinia amylovora* (Steinberger and Beer 1988; Barny et al. 1990; Walters et al. 1990; Bauer and Beer 1991), and several other pathovars of *P. syringae* (e.g., Huang et al. 1988; Lindgren et al. 1988; Fig. 1C). In addition, genes with DNA homology, and in some cases functional homology, have been isolated from other species, e.g., the so-called *wts* genes from *E. stewartii* (Coplin et al. 1992; Laby and Beer 1992), and a region containing pathogenicity genes from *X.c.* pv. *glycines* that complement *hrp* mutants of *X.c.* pv. *vesicatoria* (Hwang et al. 1992; Bonas, unpublished results). Interestingly, nonpathogenic xanthomonads that were originally isolated from diseased plants as opportunists together with pathogenic bacteria do not contain *hrp*-related DNA sequences (Stall and Minsavage 1990; Bonas et al. 1991). In *Agrobacterium tumefaciens* or in strains of *Rhizobium* ssp. there seem to be no *hrp* gene equivalents present (Bonas et al. 1991; Laby and Beer 1992). This conclusion is based on DNA hybridization experiments and, of course, does not exclude the presence of genes with functional homology to *hrp* genes in these species.

In all of the cases mentioned above, the *hrp* genes are organized in clusters of 22–40 kb, and I will restrict most of this chapter to these large *hrp* clusters. In addition, several smaller *hrp* loci have been described that are not linked to the large cluster present in the same bacterium. These include a region in *P. solanacearum* (Huang et al. 1990), the *hrpX* locus that is conserved in *X. campestris* pathovars *campestris* (Kamoun et al. 1993, and the *hrpM* locus in *P.s.* pv. *syringae* (Niepold et al. 1985; Mukhopadhyay et al. 1988). *hrpM* is functionally conserved in pathovar *phaseolicola* (Fellay et al. 1991). Besides being nonpathogenic and unable to induce the HR in tobacco, *P. syringae hrpM* mutants are also affected in mucus production. The *hrpM* locus encodes two putative proteins which are similar and have been shown to be functionally homologous to the products of the *E. coli mdoGH* operon (Loubens et al. 1993). The *mdoGH* genes are required for periplasmic membrane-derived oligosaccharide synthesis in *E. coli*. The *hrpQ* and *hrpT* genes from *P.s.* pv. *phaseolicola* (Miller et al. 1993) will be discussed later in this chapter.
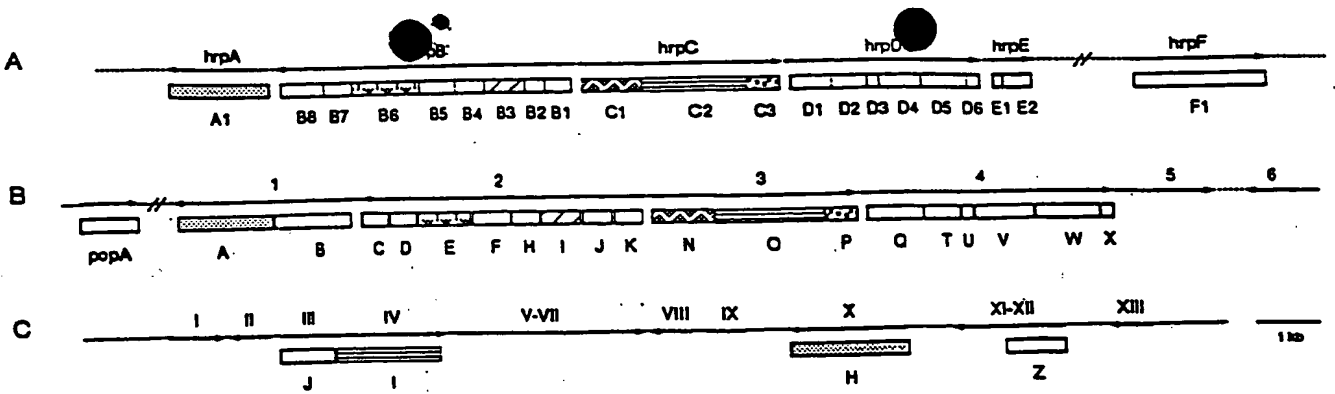
Fig. 1A–C. Genetic and translational organization of the *hrp* gene cluster of different plant pathogenic bacteria. **A** *Xanthomonas campestris* pv. *vesicatoria*; **B** *Pseudomonas solanacearum*; and **C** *Pseudomonas syringae* pv. *syringae*. Arrows represent transcription units as determined by genetic analyses. Boxes correspond to sequences of open reading frames (ORFs) that have been published. In case of sequence similarities between ORFs in different clusters the boxes are filled with the same pattern. For references, see text

3 Structural Organization and Relatedness of hrp Clusters

Genetic studies using transposon-induced insertion mutants in the respective bacterial wild-type strains revealed that the *hrp* clusters contain at least six to eight complementation groups (Fig. 1). Some *hrp* gene clusters have clearly been shown to be localized in the chromosome, e.g., in *P.s.* pv. *phaseolicola* (RAHME et al. 1991) and in *X.c.* pv. *vesicatoria* (BONAS et al. 1991), whereas in *P. solanacearum*, the *hrp* cluster is on a megaplasmid (BOUCHER et al. 1987).

Striking similarities have recently been found between the *hrp* genes of pathogens belonging to different genera. The first indication of homologies came from Southern hybridization studies. DNA homology was observed among different strains of the same pathovar, as well as between pathovars or strains within a species, and in some cases also between species. However, the degree of conservation varies. DNA homology is high within pathovars of a given subspecies, e.g., in *P. syringae* (LINDGREN et al. 1988; HUANG et al. 1991 and in *X. campestris* (BONAS et al. 1991). The latter studies were recently extended by PCR using primers based on *hrp* sequences from *X.c.* pv. *vesicatoria* (LEITE et al. 1994). Furthermore, at least some regions of the *hrp* clusters appear to be conserved on the DNA level between *P. solanacearum* and pathovars of *X. campestris*, *P. syringae*, and also to *E. amylovora* (BOUCHER et al. 1987; ARLAT et al. 1991; GOUGH et al. 1992; LABY and BEER 1992). In addition, cross-complementation within a subspecies indicated a high degree of functional conservation of *hrp* genes (e.g., LINDGREN et al. 1988; ARLAT et al. 1991; BONAS et al. 1991; LABY and BEER 1992). Due to sequence data it is now becoming more and more apparent that several *hrp* genes are conserved in all major gram-negative plant pathogenic bacteria (see below). Whether there are *hrp* genes that are clearly pathovar-specific can only be answered when complete sequence information becomes available for several *hrp* clusters.

4 Function of hrp Genes in Xanthomonas campestris pv. vesicatoria and Other Plant Pathogenic Bacteria

DNA sequence analysis of the *hrp* genes has revealed some important clues to their possible biochemical functions. One of the first genes sequenced was a regulatory gene, *hrpS*, from *P.s.* pv. *phaseolicola* (GRIMM and PANOPOULOS 1989). This gene as well as *hrpB*, a regulatory gene from *P. solanacearum* (GENIN et al. 1992), will be discussed below in the context of gene regulation.

Since *hrp* genes are environmentally regulated (see below), it was believed for a while that they would be encoding "alternative" proteins required for adaptation of the bacterium to the plant as the preferred environment. The recently discovered sequence similarities between several putative Hrp proteins and known proteins from other bacteria, however, led to a very different hypothesis, namely, involvement of Hrp proteins in protein secretion. We have

sequenced the entire hrp cluster of X.c. pv. vesicatoria. Since most hrp sequences from this and other bacteria are not yet published, I will summarize our results and refer to the other phytopathogenic bacteria as I go along. Based on genetic analyses and the open reading frames (ORFs) with a high coding probability we predict 21 hrp genes in the 25 kb hrp cluster of X.c. pv. vesicatoria. Their genetic organization is depicted in Fig. 1A. The loci hrpA and hrpB are transcribed from right to left; the other four loci are transcribed from left to right (SCHULTE and BONAS 1992a). According to the locus (hrpA-hrpF) we have numbered the ORFs consecutively. The hrpA locus appears to contain just one hrp gene, hrpA1. The hrpB operon contains eight ORFs, called hrpB1-hrpB8, etc. A region of about 4 kb between hrpE and hrpF does not seem to be involved in the interaction with the plant because insertions in this region do not lead to a change in phenotype (BONAS et al. 1991).

What are the characteristics of the Hrp proteins? It should be noted that, except for three proteins, expression of the other 18 has yet to be demonstrated in X.c. pv. vesicatoria. A number of putative Hrp proteins are most likely associated with or localized in the bacterial membrane. For example, the HrpC2 protein sequence contains eight transmembrane domains but lacks a signal sequence, suggesting an inner membrane localization (FENSELAU et al. 1992). Both HrpA1 and HrpB3 contain an NH_2-terminal signal sequence and one (HrpA1) or two (HrpB3) transmembrane domains, suggesting that a part of these proteins might be targeted to the outer membrane. The signal sequence of HrpB3 resembles signal peptidase II sequences which are typical of lipoproteins (FENSELAU et al. 1992). Experiments using radioactively labeled palmitate are underway to test whether HrpB3 is a lipoprotein. Recently, both HrpB3 and HrpA1 were shown to be localized in the X.c. pv. vesicatoria membrane fraction using polyclonal antibodies (S. Fenselau, C. Marie, and U. Bonas, manuscript in preparation). The HrpB6 protein is a putative ATPase with highly conserved nucleotide and magnesium binding domains. It is more similar to protein traffic ATPases than to proton pump ATPases, and the lack of membrane spanning domains suggests a cytoplasmic location (FENSELAU et al. 1992).

Searches of the database revealed sequence relatedness of more than half of the X.c. pv. vesicatoria Hrp proteins with putative proteins in other bacteria, including different plant pathogens. High DNA sequence identity (more than 90%) was found to a 2.7 kb fragment carrying pathogenicity genes from X.c. pv. glycines (HUANG et al. 1992). The authors predicted two ORFs, whereas in X.c. pv. vesicatoria, this region contains three ORFs corresponding to the hrpC3, hrpD1 and hrpD2 genes. Complementation studies indicated that part of the hrp region is colinear in the two pathovars of Xanthomonas (unpublished).

The deduced amino acid sequences of hrp genes published from P. solanacearum (GOUGH et al. 1992, 1993; GENIN et al. 1992) show significant similarity to X.c. pv. vesicatoria proteins (Table 1; Fig. 1). One exception is the hrpB regulatory gene from P. solanacearum which is not present in the 25 kb hrp region or in the flanking region of the X.c. pv. vesicatoria hrp cluster as determined by DNA sequence analysis and hybridization studies (T. Hoins and U. Bonas,

unpublished). Furthermore, several of the proteins mentioned are conserved in other species (Fig. 1), however, the degree of sequence similarity varies greatly (Table 1). The HrpA1 protein fr m X.c. pv. vesicatoria is 48% and 29% identical to proteins from P. solanacearum (HrpA; GOUGH et al. 1992) and P.s. pv. syringae (HrpH; HUANG et al. 1992), respectively. HrpC2 from X.c. pv. vesicatoria is even more conserved, being 66% identical to the corresponding HrpO protein of P. solanacearum (GOUGH et al. 1993), whereas the hrp genes from E. amylovora (WEI and BEER 1994) and from P.s. pv. syringae (HUANG et al. 1993) both show 62% similarity to hrpC from X.c. pv. vesicatoria. P.s. pv. syringae also contains a hrpB3 related gene, called hrpY, and a hrpD2 related gene, hrpW (H.-C. Huang, personal communication). Thus, the high degree of DNA sequence conservation that was reported earlier (see above) is also seen on the protein level. It appears that hrp genes in X.c. pv. vesicatoria are more and more closely related to P. solanacearum than to P. syringae and to Erwinia. As more and more homologous hrp genes are found in other bacteria nomenclature might become confusing. However, as long as the genes have not been shown to be functionally homologous, we will continue to use these names.

Besides genes that are conserved among the major phytopathogenic bacteria some genes are absent in the hrp region of more distantly related species. For example, there are no known homologs of the harpin genes hrpN (WEI et al. 1992a), and hrpZ (HE et al. 1993) (see below), and of hrpJ from P.s. pv. syringae (HUANG et al. 1993) in the X.c. pv. vesicatoria hrp cluster (unpublished; see Fig. 1).

Similarities of 50%-60% were found recently between HrpA1 and HrpB3 from X.c. pv. vesicatoria and two putative Nol proteins of Rhizobium fredii that are encoded by a cultivar specificity region. NolT and NolW mutants have a wider host range in nodulation of soybean (MEINHARDT et al. 1993). In addition, the authors mention that release of proteins is affected.

Last but not least, Table 1 summarizes the significant sequence similarities which have been found to proteins from animal bacterial pathogens. A number of putative Hrp proteins are related to proteins in animal pathogens such as Salmonella, Shigella, and Yersinia ssp. Since the first similarities found were to the Ysc, Vir, and Lcr proteins from Yersinia ssp, this group of organisms became a "role model" for plant pathologists (FENSELAU et al. 1992; GOUGH et al. 1992; HUANG et al. 1992). In Yersinia, these proteins are essential for the secretion of virulence factors, called Yops (Yersinia outer protein; MICHIELS et al. 1990, 1991). Since they are described in detail in the chapter by G.R. Cornelis, I will mention only a few important features. The Yops are hydrophilic proteins that lack a typical NH_2-terminal signal peptide, and are secreted by using an entirely different pathway from that previously described for protein secretion. The genes involved in secretion are clustered on a 70 kb virulence plasmid. In case of a mutation, e.g., in Ysc, the Yops accumulate in the cytoplasm (MICHIELS et al. 1991). Although their direct role in transport has yet to be demonstrated, it is believed that the Ysc and Lcr proteins mentioned in Table 1 are parts of a special transport apparatus for Yop secretion. Similarly, Shigella flexneri secretes virulence factors, called Ipa (invasion plasmid antigens), that are distinct from Yops but share the general

Table 1. Sequence similarities of *Xanthomonas campestris* pv. *vesicatoria* Hrp proteins

Xanthomonas campestris pv. vesicatoria	HrpA1[1]	HrpB6[1]	HrpB3[1]	HrpC1[2]	HrpC2[1]	HrpC3[2]	HrpD1[2]	HrpD2[2]
Pseudomonas solanacearum	HrpA[3] (66%)	HrpE[3]	HrpI[3] (70%)	HrpN[3] (74%)	HrpO[3] (81%)	HpaP[3] (54%)	HrpQ[4]	HrpT[4]
Pseudomonas syringae pv. syringae	HrpH[6] (52%)				HrpI[7] (62%)			
Yersinia enterocolitica	YscC[8] (55%)		YscJ[8] (56%)					
Yersinia pestis	YscC[8] (55%)				LcrD[10] (70%)		LsaA[11] (52%)	LsaB[11] (72%)
Yersinia pseudotuberculosis		YscN[12] (73%)	LcrKa[13] (56%)					
Shigella flexneri	MxiD[14] (50%)	Spa47[15] (65%)	MxiJ[16] (52%)	Spa40[17] (55%)	MxiA[18] (65%)			Spa24[19] (67%)
Salmonella typhimurium	InvG[19] (52%)	SpaL[21] (70%) FliI[21] (65%)		SpaS[20] (56%)	InvA[22] (67%)			SpaP[20] (64%)
Bacillus subtilis		FlaA-ORF4[23] (68%)		FlhB[24] (62%)	FlhA[25] (63%)			FliP[26] (68%)
Escherichia coli		β-F1[27] (53%)						FliP[28] (65%)
Erwinia carotovora							MopB[29] (49%)	MopC[29] (65%)
Erwinia amylovora					HrpI[30] (62%)			
Rhizobium fredii	NolW[31] (51%)		NolT[2] (61%)					
Caulobacter crescentus					FlbF[32] (55%)			

Similarities between deduced amino acid sequences of Hrp proteins from *X.c.*pv.*vesicatoria* and other proteins include conservative amino acid exchanges. Number in parentheses indicates percent similarity.

Superscript numbers indicate references as follows:
1, FENSELAU et al. 1992; 2.Bonas et al., unpublished; 3. GOUGH et al. 1992; 4, GENIN et al. 1993, sequences unpublished; 5. GOUGH et al. 1993; 6. HUANG et al. 1992; 7, HUANG et al. 1993; 8. MICHIELS et al. 1991; 9. HADDIX and STRALEY 1992; 10. PLANO et al. 1991; 11, Fields et al. unpublished, accession # L22495; 12. Galyov, unpublished, accession # U00998; 13. RIMPILAINEN et al. 1992; 14. ALLAOUI et al. 1993; 15. VENKATESAN et al. 1992; 16. ALLAOUI et al. 1992; 17. SASAKAWA et al. 1993; 18. ANDREWS and MAURELLI 1992; 19. Lodge et al.. unpublished, accession # X75302; 20. GROISMAN and OCHMAN 1993; 21. VOGLER et al. 1991; 22. GALÁN et al. 1992; 23. ALBERTINI et al. 1991; 24. Carpenter et al.. unpublished, accession # X741212; 25. CARPENTER and ORDAL 1993; 26. BISCHOFF et al. 1992; 27. SARASTE et al. 1981; 28. MALAKOOTI et al. unpublished, accession # L21994; 29. MULHOLLAND et al. 1993; 30. WEI and BEER 1993; 31. MEINHARDT et al. 1993; 32. RAMAKRISHANAN et al. 1991; SANDERS et al. 1992.
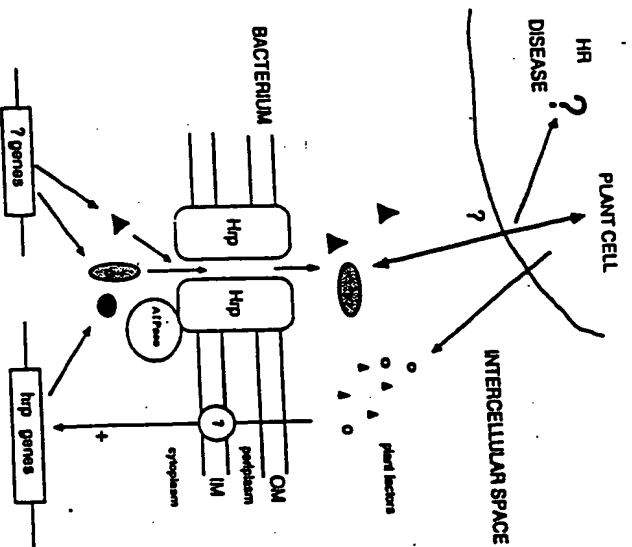
Fig. 2. Hypothetical model of cell signaling between gram-negative bacteria and plants indicating the proposed function of Hrp proteins as an apparatus for protein secretion. The model has been modified after FENSELAU et al. (1992). Hrp proteins may form a tunnel that enables the export of molecules such as virulence factors or avirulence factors leading to either a hypersensitive response (HR) or disease. These factors could be encoded by hrp genes or genes unlinked to the large cluster. Both types of genes have been found to encode elicitors of the HR (see text). The secretion of virulence proteins is hypothetical

features mentioned above (HALE 1991; and see chapter by PANSU, this volume). Although S. typhimurium appears to possess a secretion system similar to that in Shigella, secreted invasion antigens have not yet been identified (GALÁN and OCHMAN 1993; see chapter by FINLAY). As unpublished reports indicate that more and more genes in the animal pathogens are conserved, the data shown in Table 1 will soon be out of date. Proteins from other bacteria, e.g. E. coli, Bacillus, Caulobacter and from the nnop region in E. carotovora (MULHOLLAND et al. 1993), have also been found to be similar to Hrp proteins (Table 1). Most of these are important for the assembly of the flagella, motility, or chemotaxis, again pointing, in my opinion, to a specialized secretion system rather than an involvement of hrp genes in chemotaxis.

These observations led us and others to propose a hrp-dependent secretion system in plant pathogenic bacteria (FENSELAU et al. 1992; GOUGH et al. 1992; VAN GIJSEGEM et al. 1993). A model is shown in Fig. 2 and raises certain questions, e.g. if secretion occurs, what is being secreted by plant pathogenic bacteria? So far, a few proteins have been identified as elicitors of the HR but there is no evidence for secretion of virulence factors (see below).

5 hrp-dependent Secretion of Hypersensitive Response-Inducing Proteins

5.1 Harpin from Erwinia amylovora

An important feature of the isolated hrp clusters from both E. amylovora and P.s. pv. syringae is the ability of E. coli or Pseudomonas fluorescens transformants containing the cloned genes to induce the HR on tobacco (HUANG et al. 1988; BEER et al. 1991; see below). This has prompted to search for the HR-inducing activity within the respective gene clusters.

The first bacterial HR-inducing protein identified, designated harpin, is a cell envelope-associated protein encoded by the hrpN gene of E. amylovora, a pathogen of pear and apple (WEI et al. 1992a). This harpin is a glycine-rich and heat-stable protein that induces the HR in the non-host tobacco. The hrpN gene is localized within the respective hrp cluster and thus has a dual role in also being required for pathogenicity on the normal host plant. Its function in pathogenicity, however, is unknown. BEER et al. (1993) mentioned in a preliminary report that the hrpN gene seems to be conserved among Erwinia ssp. but that there is no DNA homology between hrpN and sequences in the other plant pathogenic bacteria. Although data described below suggest that the harpin protein might be secreted via the Hrp secretory apparatus, there is no published information available that demonstrates this.

5.2 Harpin from Pseudomonas syringae pv. syringae

Using an elegant approach He and coworkers recently have identified harpin, which is encoded by the hrpZ gene in the bean pathogen P.s. pv. syringae (HE et al. 1993; see Fig. 1C and chapter by Collmer and Bauer). Lysates of E. coli clones containing an expression library, made using the cloned P.s. pv. syringae hrp cluster, were directly screened for HR-inducing activity on tobacco leaves. Two proteins were identified, one of which was an NH_2-terminal deletion of harpin, with even higher activity than the full size protein. Whether or not processing occurs in natural infection is not clear. Interestingly, two short direct repeats in the COOH-terminus of harpin are essential for elicitor activity. Although the two harpins harpin, and harpin differ in their primary sequence, they have several features in common, e.g. a stretch of 22 amino acid that is similar in both proteins (HE et al. 1993). Harpin is also glycine-rich and heat-stable. As with harpin of E. amylovora, the function of harpin in pathogenicity is unknown. Its product is secreted by P.s. pv. syringae in a HrpH-dependent way; HrpH is highly related to proteins involved in secretion in other plant and animal pathogens (HUANG et al. 1992; see Table 1).

5.3 PopA from Pseudomonas solanacearum

An HR-inducing protein has been identified and characterized from P. solanacearum culture supernatants, called Pop (Pseudomonas out protein; ARLAT et al. 1994). PopA1 and two shorter derivatives, PopA2 and PopA3, induce the HR in tobacco and in certain, but not all, Petunia lines. Like the harpins, the Pop proteins are also heat-stable and glycine-rich, however, the sequence is entirely different. In contrast to the harpins, the popA gene is not a hrp gene but is located outside of the large hrp cluster. Interestingly, expression of popA is hrpB-dependent, i.e., the gene is part of the hrp regulon. Mutations in popA do not affect the HR on tobacco or pathogenicity on tomato suggesting that more than one HR-inducing factor is produced. ARLAT et al (1994) convincingly showed that secretion of PopA is dependent on other hrp genes, such as hrpA, hrpN, and hrpO (Fig. 1B). If a bacterial strain virulent towards Petunia is found it will be interesting to see if PopA acts as an avirulence protein in Petunia as has been suggested by the authors.

These exciting findings prove that certain Hrp proteins of P.s. pv. syringae and P. solanacearum play a role in transport of HR elicitors (Fig. 2). They also stimulate more questions. It needs to be shown that harpins and PopA are in fact secreted when the bacteria interact with the plant (the hrp genes were induced in vitro). Are harpins conserved among pathovars of P. syringae? How many elicitors f the n n-host HR in tobacco can be found? Is the mechanism of recognition in tobacc identical with the Erwinia and P.s. pv. syringae harpins and the P. solanacearum Pops?

6 Regulation of Expression of hrp Genes

Expressi n of hrp genes is controlled by environmental conditions and has been studied on the RNA levels as well as using transcriptional fusions to reporter genes such as the E. coli genes encoding β-galactosidase or β-glucuronidase. In general, expression of hrp loci is not detectable when the bacteria are grown in complex culture media. However, after growth of the bacteria in the plant, hrp genes are expressed. Attempts to mimic the conditions that the different bacterial species encounter in the plant tissue resulted in the finding that growth in minimal media without any plant-derived factor was sufficient to induce hrp genes. This has led to the speculation that the bacteria have to experience some kind of starvation conditions for full expression of hrp genes. One of the first indications for hrp gene expression in vitro, and clearly a breakthrough, was a report on the hrp-dependent expression of an avirulence gene from the soybean pathogen P.s. pv. glycinea (HUYNH et al. 1989).

Since the composition of minimal media differs depending on the bacterium studied, the most important findings will be summarized for representative pathogens. Parameters like carbon source, concentration of organic nitrogen and phosphate, osmolarity, and pH have been found to be important. High con-

centration of organic nitrogen generally appears to suppress hrp gene activation. Only two regulatory genes have been studied so far (see below). Interestingly, they both belong to different families of regulatory proteins.

6.1 Pseudomonas syringae

Expression of all seven hrp loci in the large cluster of P.s. pv. phaseolicola is suppressed in complex medium but induced in the plant. Induction occurs in the susceptible host plant as well as in the non-host, tobacco; suggesting that there is no plant species-specific molecule involved in control of host range (RAHME et al. 1992). Five complementation groups, hrpAB, hrpC, hrpD, hrpE and hrpF, can also be induced in M9 minimal medium containing sucrose as a carbon source, however, induction is affected by pH, osmolarity, and carbon source, and never reaches the levels obtained in the plant (RAHME et al. 1992). A similar observation was made earlier for the avirulence gene avrB in P.s. pv. glycinea. Induction occurred in a minimal medium containing fructose, mannitol, or sucrose. Expression of avrB is dependent on hrp genes homologous to hrpRS and hrpL from P.s. pv. phaseolicola and was suppressed by TCA cycle intermediates such as citrate and succinate (HUYNH et al. 1989). hrp gene expression in P.s. pv. syringae occurs in the same medium as described by HUYNH et al. (1989); (HUANG et al. 1991; XIAO et al. 1992). The authors showed hrp gene induction in the non-host plant, tobacco, but no data for the host plant. The P.s. pv. phaseolicola loci hrpL and hrpRS are only expressed to a very low level in M9 minimal medium and are induced at least 1000-fold when the bacteria are inoculated into the plant. This led to the conclusion that, at least for expression of hrpL and hrpRS, specific plant factors might be necessary (RAHME et al. 1992).

6.2 Regulatory Genes hrpRS and rpoN of Pseudomonas syringae pv. phaseolicola

The results on environmental factors inducing or suppressing hrp gene expression suggested that specific regulatory genes are involved in the control of hrp promoter activities. At least two loci are involved in positive regulation of the other hrp loci of P.s. pv. phaseolicola hrp cluster (FELLAY et al. 1991). While there is no information published for hrpL, hrpRS has been sequenced. It contains two genes whose predicted protein products are 60% identical to each other (GRIMM and PANOPOULOS 1989; MILLER et al. 1993). The HrpS protein is similar to members of the NtrC family of regulatory proteins in gram-negative bacteria. Most NtrC-like regulatory proteins are members of two-component systems, with a sensor protein that in turn activates a response element by phosphorylation of a site in the conserved NH_2-terminal domain (ALBRIGHT et al. 1989). The putative sensor component operating in hrp gene regulation has not been identified. It is postulated that HrpS is the activating protein, however, direct biochemical data

have not been presented. The lack of a typical NH$_2$-terminal domain in HrpS could indicate that a different mechanism may be involved in HrpS activation. Apparently, hrpS-related sequences are also present in other bacteria, e.g., in P.s. pv. syringae (HEU and HUTCHESON 1993) and in Erwinia amylovora (BEER et al. 1993). E. stewartii contains a transcriptional regulator, WtsA, with 52% identity to HrpS of P.s. pv. phaseolicola. The HrpS clone, however, was unable to functionally complement a wtsA mutant (FREDERICK et al. 1993).

The structure of the hrpRS locus and the finding of –24/–12 consensus sequences upstream of hrpRS indicated a possible role in transcriptional activation for transcription factor sigma 54, encoded by rpoN (GRIMM and PANOPOULOS 1989). In a preliminary report, FELLEY et al. (1991) demonstrated that hrp gene expression in P.s. pv. phaseolicola is indeed dependent on rpoN. A rpoN mutant of P.s. pv. phaseolicola is a glutamine auxotroph and nonpathogenic. Whether rpoN is generally involved in regulation of hrp gene expression is not clear. In X.c. pv. vesicatoria, rpoN is clearly not involved in hrp gene expression and pathogenicity (T. Horns and U. Bonas, manuscript in preparation).

Recently, MILLER et al. (1993) have reported the identification of two new loci, hrpQ and hrpT, from P. s. pv. phaseolicola that affect activation of hrpRS in trans. However, since hrpRS is strongly induced in plants while both hrpQ and hrpT are constitutively expressed, there must be more factors involved in hrp gene regulation. Strains carrying mutations in either hrpQ or hrpT are amino acid auxotrophs (methionine and tryptophan). hrpQ and hrpT are probably involved in methionine and tryptophan biosynthesis, respectively (MILLER et al. 1993). As stated above, such mutants would normally have been eliminated from the hrp minimal analysis.

6.3 Conserved Sequence Boxes in *Pseudomonas syringae*

A conserved sequence, the so-called harp box (TG(A/C)AANC, FELLEY et al. 1991), upstream of four hrp loci in P. s. pv. phaseolicola, was suggested to be involved in positive regulation of expression. Similar motifs were described for the promoter regions of several P. syringae avirulence genes, the expression of which is dependent on hrpRS and on rpoN (HUYNH et al. 1989; SALMERON and STASKAWICZ 1993; INNES et al. 1993; SHEN and KEEN 1993). These studies led to a revised 'harp' box sequence (GGAACCNA). Its significance in protein binding has not been shown but avrD promoter constructs lacking the harp box are no longer inducible (SHEN and KEEN 1993). A harp box-related motif was also found upstream of transcription unit 3 in P. solanacearum (GOUGH et al. 1993).

There is no harp box sequence in Xanthomonas hrp gene promoters. Another sequence motif that occurs in the promoter region of hrp loci in X. c. pv. vesicatoria was recently identified. This "PIP" (plant-inducible promoter) box has the sequence TTCGC-N15-TTCGC and occurs upstream of the –35 consensus sequence in four out of six hrp promoters (S. Fenselau and U. Bonas, unpublished). Experiments are underway to test whether this is a protein binding motif.

6.4 *Xanthomonas campestris*

Expression of hrp genes in X. c. pv. campestris was determined after growth in vitro and found to be induced in a minimal medium with sucrose and/or fructose as carbon source. No expression occurred in complex media or with high concentrations of organic nitrogen (ARLAT et al. 1991). In X. c. pv. vesicatoria, expression of the six hrp loci is induced in the plant but cannot be efficiently induced in the synthetic media tested so far. However, culture filtrates of sterile tomato cell suspension cultures (called TCM) induced expression of the six hrp loci in X. c. pv. vesicatoria whereas the basal Murashige-Skoog culture medium did not. The inducing factor(s) could only partially be purified from TCM and was found to be smaller than 1000 dalton, heat-stable, organic, and hydrophilic (SCHULTE and BONAS 1992a). De novo transcription of all hrp loci occurs rapidly within 1 h after transfer of the bacteria into TCM (S. Fenselau and U. Bonas, unpublished). A minimal medium was designed which would not suppress hrp gene induction. This medium, called XVM1, induces the hrpF locus (Fig. 1A) to high levels and differs from the other media described above, in particular by its low concentration in phosphate. Both sucrose and methionine are needed for efficient induction. It is not known whether a plant factor is necessary for activation of the other hrp loci, or if the XVM1 medium still lacks components or contains them in suppressing amounts (SCHULTE and BONAS 1992b).

6.5 *Erwinia and Pseudomonas solanacearum*

The hrp genes of Erwinia amylovora are rapidly induced in the non-host, tobacco, and more slowly in the host, pear. Several loci were induced in minimal medium with mannitol as a carbon source. Induction was suppressed by high concentrations of nitrogen and by glucose and was slightly temperature dependent (WEI et al. 1992b).

In P. solanacearum, the hrp cluster was also induced in host and in non-host plants, as well as in minimal medium. The best carbon sources for induction of four of the six transcription units were pyruvate and glutamate while, as in other bacteria, casamino acids suppressed induction (ARLAT et al. 1992). The two rightmost hrp transcription units (5 and 6; Fig. 1B) are constitutively expressed but can be induced under certain conditions (GENIN et al. 1992).

The only other gene reported to regulate hrp gene expression is hrpB from P. solanacearum. The gene is part of the hrp cluster and appears to be a member of the AraC family of positive regulatory proteins. Interestingly, hrpB is related to virF of Yersinia (CORNELIS et al. 1989; GENIN et al. 1992). The hrpB gene positively regulates four of the six hrp loci, as well as the popA locus, located outside of the hrp cluster which encodes a protein secreted in a Hrp-dependent way (see above; ARLAT et al. 1994). Whether the HrpB protein binds directly to hrp promoters is not yet known.

At this time one can only speculate whether the regulatory systems for hrp gene expression employed by P. solanacearum and P. syringae are really different

or whether there is a global regulatory network thus allowing the fine tuning of gene expression in response to environmental cues. In conclusion, most *hrp* loci from different bacteria are inducible in a particular minimal medium. At this time it cannot be ruled out that stimulation of *hrp* gene expression involves specific plant factors as was described for the virulence genes of *Agrobacterium* (WINANS 1992). Since the composition of the nutrients available to the pathogen in the plant is not known one can only speculate that the conditions described above reflect the situation in the plant. It is noteworthy that the in vitro culture will only mimic the dynamic nutritional situation that bacteria experience in their interaction with a plant for a short time. In mammalian bacterial pathogens, the expression of genes involved in virulence is also regulated in response to environmental cues rather than to specific host molecules. This subject has been reviewed recently (MEKALANOS 1992 and in accompanying chapters), and I will only mention some important factors. In *Yersinia*, the *vir* and *lcr* genes are regulated by low calcium (low calcium response genes; STRALEY et al. 1993) and by temperature (CORNELIS et al. 1989; see chapter by CORNELIS). A calcium effect has not been described for any plant bacterium. In our laboratory no effect of calcium on *hrpF* gene expression in XVM1 was observed (SCHULTE and U. BONAS, unpublished). Expression of *invA* of *S. typhimurium* of the *mxi* and *ipa* genes of *Shigella* is affected by osmolarity and the later genes also by temperature (GALÁN and CURTISS 1990; HALE 1991).

R f rences

Albertini AM, Caramori T, Crabb WD, Scoffone F, Galizzi A (1991) The llaA locus of *Bacillus subtilis* is part of a large operon coding for flagellar structures, motility functions, and an ATPase-like polypeptide. J Bacteriol 173 3573-3579

Albright LM, Huala E, Ausubel FM (1989) Prokaryotic signal transduction mediated by sensor and regulator protein pairs. Annu Rev Genet 23: 311-336

Allaoui A, Sansonetti PJ, Parsot C (1992) MxiJ, a lipoprotein involved in secretion of *Shigella* Ipa invasins, is homologous to YscJ, a secretion factor of the *Yersinia* Yop proteins. J Bacteriol 174: 7661-7669

Allaoui A, Sansonetti PJ, Parsot C (1993) MxiD, an outer membrane protein necessary for the secretion of the *Shigella flexneri* Ipa invasins. Mol Microbiol 7: 59-68

Andrews GP, Maurelli AT (1992) mxiA of *Shigella flexneri* 2a, which facilitates export of invasion plasmid antigens, encodes a homolog of the low-calcium response protein, LcrD, of *Yersinia pestis*. Infect Immun 60: 3287-3295

Arlat M, Gough CL, Barber CE, Daniels MJ (1991) *Xanthomonas campestris* contains a cluster of *hrp* genes related to the *hrp* cluster of *Pseudomonas solanacearum*. Mol Plant Microbe Interact 4: 593-601

Arlat M, Gough CL, Zischek C, Barberis PA, Trigalet A, Boucher CA (1992) Transcriptional organization and expression of the large *hrp* gene cluster of *Pseudomonas solanacearum*. Mol Plant Microbe Interact 5: 187-193

Arlat M, Van Gijsegem F, Huet JC, Pernollet JC, Boucher CA (1994) PopaA1, a protein which induces a hypersensitive-like response on specific *Petunia* genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*. EMBO J 13: 543-553

Barny MA, Guinebretière MH, Marçais B, Coissac A, Paulin JP, Laurent J (1990) Cloning of a large gene cluster involved in *Erwinia amylovora* CFBP1430 virulence. Mol Microbiol 4: 777-787

Bauer DW, Beer SV (1991) Further characterization of an *hrp* gene cluster of *Erwinia amylovora*. Mol Plant Microbe Interact 4: 493-499

Beer SV, Bauer DW, Jiang X, Laby RJ, Sneath BJ, Wei ZM, Wilcox DA, Zumoff CH (1991) The *hrp* gene cluster of *Erwinia amylovora*. In: Hennecke H, Verma DPS (eds) Advances in molecular genetics of plant-microbe interactions, vol 1, Kluwer Academic, Dordrecht, Netherlands, pp 53-60

Beer SV, Wei ZM, Laby RJ, He SY, Bauer DW, Collmer A, Zumoff C (1993) Are harpins universal elicitors of the hypersensitive response of phytopathogenic bacteria? In: Nester EW, Verma DPS (eds) Advances in molecular genetics of plant-microbe interactions vol 2, Kluwer Academic, Dordrecht, Netherlands, pp 281-286

Bischoff DS, Weinreich MR, Ordal GW (1992) Nucleotide sequence of *Bacillus subtilis* flagellar biosynthetic genes *fliP* and *fliQ* and identification of a novel flagellar gene. J Bacteriol 174: 4017-4025

Bonas U, Schulte R, Fenselau S, Minsavage GV, Staskawicz BJ, Stall RE (1991) Isolation of a gene cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. Mol Plant Microbe Interact 4: 81-88

Boucher CA, Van Gijsegem F, Barberis PA, Arlat M, Zischek C (1987) *Pseudomonas solanacearum* genes controlling both pathogenicity and hypersensitivity on tobacco are clustered. J Bacteriol 169: 5626-5632

Boucher CA, Gough CL, Arlat M (1992) Molecular genetics of pathogenicity determinants of *Pseudomonas solanacearum* with special emphasis on *hrp* genes. Annu Rev Phytopathol 30: 443-461

Carpenter PB, Ordal GW (1993) *Bacillus subtilis* FlhA: a flagellar protein related to a new family of signal-transducing receptors. Mol Microbiol 7: 735-743

Coplin DL, Frederick RD, Majerczak DR, Tuttle LD (1992) Characterization of a gene cluster that specifies pathogenicity in *Erwinia stewartii*. Mol Plant Microbe Interact 5: 81-88

Cornelis G, Sluiters C, Lambert de Rouvroit C, Michiels T (1989) Homology between VirF, the transcriptional activator of the *Yersinia* virulence regulon, and AraC, the *Escherichia coli* arabinose operon regulator. J Bacteriol 171: 254-262

Fellay R, Rahme LG, Mindrinos MN, Frederick RD, Pisi A, Panopoulos NJ (1991) Genes and signals controlling the *Pseudomonas syringae* pv. *phaseolicola*-plant interaction. In: Hennecke H, Verma DPS (eds) Advances in molecular genetics of plant-microbe interactions vol 1. Kluwer Academic, Dordrecht, Netherlands, pp 45-52

Fenselau S, Balbo I, Bonas U (1992) Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in secretion in bacterial pathogens of animals. Mol Plant-Microbe Interact 4: 593-601

Frederick RD, Majerczak DR, Coplin DL (1993) *Erwinia stewartii* WtsA, a positive regulator of pathogenicity gene expression, is similar to *Pseudomonas syringae* pv. *phaseolicola* HrpS. Mol Microbiol 9: 477-485

Galán JE, Curtiss R III (1990) Expression of *Salmonella typhimurium* genes required for invasion is regulated by changes in DNA supercoiling. Infect Immun 58: 1879-1885

Galán JE, Ginocchio C, Costess P (1992) Molecular and functional characterization of the *Salmonella* invasion gene invA: homology of invA to members of a new protein family. J Bacteriol 174: 4338-4349

Genin S, Gough CL, Zischek C, Boucher CA (1992) The hrpB gene encodes a positive regulator of pathogenicity genes from *Pseudomonas solanacearum*. Mol Microbiol 6: 3065-3076

Genin S, Gough CL, Arlat M, Zischek C, Van Gijsegem F, Barberis P, Boucher CA (1993) Involvement of *Pseudomonas solanacearum hrp* genes in the secretion of a bacterial compound which induces a hypersensitive-like response on tobacco. In: Nester EW, Verma DPS (eds) Advances in molecular genetics of plant-microbe interactions, vol 2. Kluwer Academic, Dordrecht, Netherlands, pp 259-268

Goguen JD, Yother J, Straley SC (1984) Genetic analysis of the low calcium response in *Yersinia pestis* Mu d1 (Ap lac) insertion mutants. J Bacteriol 160: 842-848

Gough CL, Genin S, Zischek C, Boucher CA (1992) *hrp* genes of *Pseudomonas solanacearum* are homologous to pathogenicity determinants of animal pathogenic bacteria and are conserved among plant pathogenic bacteria. Mol Plant Microbe Interact 5: 384-389

Gough CL, Genin S, Lopes V, Boucher CA (1993) Homology between the HrpO protein of *Pseudomonas*

solanacearum and bacterial proteins implicated in a signal peptide independent secretion mechanism. Mol Gen Genet 236:378–392

Grimm C, Panopoulos NJ (1989) The predicted product of a pathogenicity locus from Pseudomonas syringae pv. phaseolicola is homologous to a highly conserved domain of several prokaryotic regulatory proteins. J Bacteriol 171:5031–5038

Groisman EA, Ochman H (1993) Cognate gene clusters govern invasion of host epithelial cells by Salmonella typhimurium and Shigella flexneri. EMBO J 12:3779–3787

Haddix PL, Straley SC (1992) Structure and regulation of the Yersinia pestis ycsBCDEF operon. J Bacteriol 174:4820–4828

Isla TL (1991) Genetic basis for virulence in Shigella species. Microbiol Rev 55:206–224

He SY, Huang HC, Collmer A (1993) Pseudomonas syringae pv. syringae harpin_ss: a protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. Cell 73:1255–1266

Huang HC, He SY, Bauer DW, Collmer A (1992) The Pseudomonas syringae pv. syringae 61 hrpH product, an envelope protein required for elicitation of the hypersensitive response in plants. J Bacteriol 174:6878–6885

Hueu S, Hutcheson SW (1993) Nucleotide sequence and properties of the hrmA locus associated with the Pseudomonas syringae pv. syringae 61 hrp gene cluster. Mol Plant Microbe Interact 6:553–564

Huang HC, Schuurink R, Denny TP, Atkinson MM, Baker CJ, Yucel I, Hutcheson SW, Collmer A (1988) Molecular cloning of a Pseudomonas syringae pv. syringae gene cluster that enables Pseudomonas fluorescens to elicit the hypersensitive response in tobacco plants. J Bacteriol 170:4748–4756

Huang Y, Xu P, Sequeira L (1990) A second cluster of genes that specify pathogenicity and host response in Pseudomonas solanacearum. Mol Plant Microbe Interact 3:48–53

Huang HC, Hutcheson SW, Collmer A (1991) Characterization of the hrp cluster from Pseudomonas syringae pv. syringae 61 and TnphoA tagging of genes encoding exported or membrane-spanning Hrp proteins. Mol Plant Microbe Interact 4:469–476

Huynh TV, Dahlbeck D, Staskewicz BJ (1989) Bacterial blight of soybean: regulation of a pathogen gene determining host cultivar specificity. Science 245:1374–1377

Hwang I, Lim SM, Shaw PD (1992) Cloning and characterization of pathogenicity genes from Xanthomonas campestris pv. glycines. J Bacteriol 174:1923–1931

Innes RW, Bent AF, Kunkel BN, Bisgrove SR, Staskewicz BJ (1993) Molecular analysis of avirulence gene avrRpt2 and identification of a putative regulatory sequence common to all known Pseudomonas syringae avirulence genes. J Bacteriol 175:4859–4869

Kamdar HV, Kamoun S, Kado CI (1993) Restoration of pathogenicity of avirulent Xanthomonas oryzae pv. oryzae and X. campestris pathovars by reciprocal complementation with the hrpXo and hrpXc genes and identification of HrpX function by sequence analyses. J Bacteriol 175:2017–2025

Kamoun S, Kado CI (1990) A plant-inducible gene of Xanthomonas campestris pv. campestris encodes an exocellular component required for growth at the host and hypersensitivity on nonhosts. J Bacteriol 172:5165–5172

Kamoun S, Kamdar HV, Tola E, Kado CI (1992) Incompatible interactions between crucifers and Xanthomonas campestris involve a vascular hypersensitive response: role of the hrpX locus. Mol Plant Microbe Interact 5:22–33

Klément Z (1982) Hypersensitivity In: Mount MS, Lacy GH (eds) Phytopathogenic prokaryotes, vol 2. Academic New York, pp 149–177

Laby RJ, Beer SV (1992) Hybridization and functional complementation of the hrp gene cluster from Erwinia amylovora strain Ea321 with DNA of other bacteria. Mol Plant Microbe Interact 5:412–419

Lambert de Rouvroit C, Sluiters C, Cornelis GR (1992) Role of the transcriptional activator, VirF, and temperature in the expression of the pYV plasmid genes of Yersinia enterocolitica. Mol Microbiol 6:395–409

Leite RP, Minsavage GV, Bonas U, Stall RE (1994) Detection and identification of plant pathogenic strains of Xanthomonas based on amplification of DNA sequences related to the hrp genes of Xanthomonas campestris pv. vesicatoria. Appl Environ Microbiol 60:1068–1077

Lindgren PB, Peet RC, Panopoulos NJH (1986) Gene cluster of Pseudomonas syringae pv. phaseolicola controls pathogenicity on bean plants and hypersensitivity on nonhost plants. J Bacteriol 168:512–522

Lindgren PB, Panopoulos NJ, Staskewicz BJ, Dahlbeck D (1988) Genes required for pathogenicity and hypersensitivity are conserved and interchangeable among pathovars of Pseudomonas syringae. Mol Gen Genet 211:499–506

Lindsay WP, Lamb CJ, Dixon RA (1993) Microbial recognition and activation of plant defense systems. Trends Microbiol 1:181–187

Loubens I, Debarbieux L, Bouin A, Lecroix J-M, Bohin J-P (1993) Homology between a genetic locus (ndoA) involved in the osmoregulated biosynthesis of periplasmic glucans in Escherichia coli and a genetic locus (hrpM) controlling pathogenicity of Pseudomonas syringae. Mol Microbiol 10:329–340

Metalanos JJ (1992) Environmental signals controlling expression of virulence determinates in bacteria. J Bacteriol 174:1–7

Meinhardt LW, Krishnan HB, Balatti PA, Pueppke SG (1993) Molecular cloning and characterization of a sym plasmid locus that regulates cultivar-specific nodulation of soybean by Rhizobium fredii USDA257. Mol Microbiol 9:17–29

Michiels T, Wattiau P, Brasseur R, Ruysschaert JM, Cornelis G (1990) Secretion of Yop proteins by Yersiniae. Infect Immun 58:2840–2849

Michiels T, Vanooteghem J-C, Lambert de Rouvroit C, China B, Gustin A, Boudry P, Cornelis GR (1991) Analysis of virC, an operon involved in the secretion of Yop proteins by Yersinia enterocolitica. J Bacteriol 173:4994–5009

Miller W, Mindrinos MS, Rahme LG, Frederick RD, Grimm C, Gressmann R, Kyriakides X, Kokkinidis M, Panopoulos NJ (1993) Pseudomonas syringae pv. phaseolicola-plant interactions: host-pathogen signalling through cascade control of hrp gene expression. In: Nester EW, Verma DPS (eds) Advances in molecular genetics of plant-microbe interactions, vol 2. Kluwer Academic, Dordrecht, pp 267–274

Mukhopadhyay P, Williams J, Mills D (1988) Molecular analysis of a pathogenicity locus in Pseudomonas syringae pv. syringae. J Bacteriol 170:5479–5488

Plano GV, Barve SS, Straley SC (1991) LcrD, a membrane-bound regulator of the Yersinia pestis low-calcium response. J Bacteriol 173:7293–7303

Mulholland V, Hinton JCD, Sidebotham J, Toth IK, Hyman LJ, Pérombelon MCM, Reeves PJ, Salmond GPC (1993) A pleiotropic reduced virulence (Rvi-) mutant of Erwinia carotovora subspecies atroseptica is defective in flagella assembly proteins that are conserved in plant and animal bacterial pathogens. Mol Microbiol 9:343–356

Neipold F, Anderson D, Mills D (1985) Cloning determinants of pathogenesis from Pseudomonas syringae pv. syringae. Proc Natl Acad Sci USA 82:406–410

Rahme LG, Mindrinos MN, Panopoulos NJ (1991) Genetic and transcriptional organization of the hrp cluster of Pseudomonas syringae pv. phaseolicola. J Bacteriol 173:575–586

Rahme LG, Mindrinos MN, Panopoulos NJ (1992) Plant and environmental sensory signals control the expression of hrp genes in Pseudomonas syringae pv. phaseolicola. J Bacteriol 174:3499–3507

Rannakrishnan G, Zhao J-L, Newton A (1991) The cell cycle-regulated flagellar gene IIbF of Caulobacter crescentus is homologous to a virulence locus (lcrD) of Yersinia pestis. J Bacteriol 173:7283–7292

Rimpiläinen M, Forsberg A, Wolf-Watz H (1992) A novel protein, LcrQ, involved in the low-calcium response of Yersinia pseudotuberculosis shows extensive homology to YopI. J Bacteriol 174:3355–3363

Salmeron JM, Staskewicz BJ (1993) Molecular characterization and hrp dependence of the avirulence gene avrPto from Pseudomonas syringae pv. tomato. Mol Gen Genet 239:6–16

Sanders LA, Van Way S, Mullin DA (1992) Characterization of the Caulobacter crescentus flbF promoter and identification of the inferred FlbF product as a homolog of the LcrD protein from a Yersinia enterocolitica virulence plasmid. J Bacteriol 174:857–866

Saraste M, Gay NJ, Eberle A, Runswick MJ, Walker JE (1981) The atp operon: nucleotide sequences of the genes for the a, b, and g subunits of Escherichia coli ATP synthase. Nucleic Acids Res 9:5287–5296

Sasakawa C, Komatsu K, Tobe T, Suzuki T, Yoshikawa M (1993) Eight genes in region 5 that form an operon are essential for invasion of epithelial cells by Shigella flexneri. J Bacteriol 175:2334–2346

Schulte R, Bonas U (1992a) Expression of the Xanthomonas campestris pv. vesicatoria hrp gene cluster, which determines pathogenicity and hypersensitivity on pepper and tomato, is plant inducible. J Bacteriol 174:815–823

Schulte R, Bonas U (1992b) A Xanthomonas pathogenicity locus is induced by sucrose and sulfur-containing amino acids. Plant Cell 4:79–86

Stein H, Kam N (1993) Characterization of the promoter of avirulence gene D from Pseudomonas syringae pv. tomato. J Bacteriol 175:5916–5924

Stall RE, Minsavage GV (1990) The use of hrp genes to identify opportunistic xanthomonads. In: Klément Z (ed) plant pathogenic bacteria, proceedings of the 7th International conference of plant pathogenic bacteria, Budapest, Hungary, 1989. Akadémiai Kiadó, Budapest, pp 369-374

Steinberger EM, Beer SV (1988) Creation and complementation of pathogenicity mutants of Erwinia amylovora. Mol Plant Microbe Interact 1: 135-144

Staley SC, Plano GV, Skrzypek E, Haddix PL, Fields KA (1993) Regulation by Ca²⁺ in the Yersinia low-Ca²⁺ response. Mol Microbiol 8: 1005-1010

Van Gijsegem F, Genin S, Boucher C (1993) Conservation of secretion pathways for pathogenicity determinants of plant and animal bacteria. Trends Microbiol 1: 175-180

Venkatesan MM, Buysse JM, Oaks EV (1992) Surface presentation of Shigella flexneri invasion plasmid antigens requires the products of the spa locus. J Bacteriol 174: 1990-2001

Vogler AP, Homma M, Iikura VM, Macnab RM (1991) Salmonella typhimurium mutants defective in flagellar filament regrowth and sequence similarity of Fil to F0F1, vacuolar, and archaebacterial ATPase subunits. J Bacteriol 173: 3564-3572

Walters K, Marcoll A, Hitchin E, Mansfield J (1990) Gene for pathogenicity and ability to cause hypersensitive reaction cloned from Erwinia amylovora. Physiol Mol Plant Pathol 36: 509-521

Weney VR, Kingsley MT, Gabriel DW (1991) Xanthomonas campestris pv. translucens genes determining host-specific virulence and general virulence on cereals identified by Tn5-gusA insertion mutagenesis. Mol Plant Microbe Interactions 4: 623-627

Wei ZM, Leby RJ, Zumoff CH, Bauer DW, He SY, Collmer A, Beer SV (1992a) Harpin, elicitor of the hypersensitive response produced by the plant pathogen Erwinia amylovora. Science 257: 85-88

Wei ZM, Sneath BJ, Beer SV (1992b) Expression of Erwinia amylovora hrp genes in response to environmental stimuli. J Bacteriol 174: 1875-1882

Wei ZM and Beer SV (1993) HrpI of Erwinia amylovora functions in secretion of harpin and is a member of a new protein family. J Bacteriol 175: 7958-7967

Willis DK, Rich JJ, Hrabak EM (1991) hrp genes of phytopathogenic bacteria. Mol Plant Microbe Interact 4: 132-138

Winans SC (1992) Two-way chemical signaling in Agrobacterium-plant interactions. Microbiol Rev 56: 12-31

Xiao YX, Lu Y, Heu SG, Hutcheson SW (1992) Organization and environmental regulation of the Pseudomonas syringae pv. syringae 61 hrp cluster. J Bacteriol 174: 1734-1741

Th Enigmatic Avirul nc G n s of Phytopathogenic Bacteria

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1 Action at the "Pathogenic Cusp"

The previous chapters have discussed how phytopathogenic bacteria can sense and respond to conditions present in a variety of microenvironments: soil, water, plant cell surfaces, and intracellular spaces. The switch from epiphyte to pathogen is apparently accompanied by fundamental reprogramming of gene activity and attendant function, as evidenced by induction of hrp genes and subsequent production of various virulence and pathogenicity factors, some of which are host-specific, some not. This reprogramming switch between epiphytic and patho-genic growth strategies, "the pathogenic cusp" (DANGL 1994), is the point at which not only the potential pathogen but also the host first sense and respond to each other. A successful plant defense response should be based on surveillance and interdiction before the pathogen has a chance to establish production of the armory of factors which determine successful colonization of that host. It is incumbent on each potential plant host, then, to evolve mechanisms to recognize some factor, preferably one produced at this pathogenic cusp, and to base resistance strategies on early recognition. Thus, an evolutionary tug-of-war is

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